



# SEQUENCE LISTING

<110> SARCABAL, PATRICIA  
CROUX, CHRISTIAN  
SOUCAILLE, PHILIPPE

<120> METHOD FOR PREPARING 1,3-PROPANEDIOL BY A RECOMBINANT  
MICRO-ORGANISM IN THE ABSENCE OF COENZYME B12 OR ONE OF  
ITS PRECURSORS

<130> CHEP:004US

<140> 10/043,639

<141> 2002-01-09

<150> PCT/FR00/01981

<151> 2000-07-07

<150> FR 99/08939

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<170> PatentIn Ver. 2.1

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40

45

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Pro Gln Val Met Phe Asn Lys Asn Leu Cys Thr Lys Cys Gly Arg Cys  
50 55 60

Lys Ser Gln Cys Lys Ser Ala Gly Ile Asp Met Asn Ser Glu Tyr Arg  
65 70 75 80

Ile Asp Lys Ser Lys Cys Thr Glu Cys Thr Lys Cys Val Asp Asn Cys  
85 90 95

Leu Ser Gly Ala Leu Val Ile Glu Gly Arg Asn Tyr Ser Val Glu Asp  
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Val Ile Lys Glu Leu Lys Lys Asp Ser Val Gln Tyr Arg Arg Ser Asn  
115 120 125

Gly Gly Ile Thr Leu Ser Gly Gly Glu Val Leu Leu Gln Pro Asp Phe  
130 135 140

Ala Val Glu Leu Leu Lys Glu Cys Lys Ser Tyr Gly Trp His Thr Ala  
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Ile Glu Thr Ala Met Tyr Val Asn Ser Glu Ser Val Lys Lys Val Ile  
165 170 175

Pro Tyr Ile Asp Leu Ala Met Ile Asp Ile Lys Ser Met Asn Asp Glu  
180 185 190

Ile His Arg Lys Phe Thr Gly Val Ser Asn Glu Ile Ile Leu Gln Asn  
195 200 205

Ile Lys Leu Ser Asp Glu Leu Ala Lys Glu Ile Ile Ile Arg Ile Pro  
210 215 220

Val Ile Glu Gly Phe Asn Ala Asp Leu Gln Ser Ile Gly Ala Ile Ala  
225 230 235 240

Gln Phe Ser Lys Ser Leu Thr Asn Leu Lys Arg Ile Asp Leu Leu Pro  
245 250 255

Tyr His Asn Tyr Gly Glu Asn Lys Tyr Gln Ala Ile Gly Arg Glu Tyr  
260 265 270

Ser Leu Lys Glu Leu Lys Ser Pro Ser Lys Asp Lys Met Glu Arg Leu  
275 280 285

Lys Ala Leu Val Glu Ile Met Gly Ile Pro Cys Thr Ile Gly Ala Glu  
290 295 300

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<211> 385

<212> PRT

<213> Clostridium butyricum

<400> 8

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Lys Ala Leu Ile Val Thr Asp Lys Phe Leu Lys Asp Met Glu Gly Gly  
35 40 45

Ala Val Glu Leu Thr Val Lys Tyr Leu Lys Glu Ala Gly Leu Asp Val  
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Val Tyr Tyr Asp Gly Val Glu Pro Asn Pro Lys Asp Val Asn Val Ile  
65 70 75 80

Glu Gly Leu Lys Ile Phe Lys Glu Glu Asn Cys Asp Met Ile Val Thr  
85 90 95

Val Gly Gly Gly Ser Ser His Asp Cys Gly Lys Gly Ile Gly Ile Ala  
100 105 110

Ala Thr His Glu Gly Asp Leu Tyr Asp Tyr Ala Gly Ile Glu Thr Leu  
115 120 125

Val Asn Pro Leu Pro Pro Ile Val Ala Val Asn Thr Thr Ala Gly Thr  
130 135 140

Ala Ser Glu Leu Thr Arg His Cys Val Leu Thr Asn Thr Lys Lys Lys

145		150		155		160
Ile Lys Phe Val	Ile Val Ser Trp Arg Asn Leu Pro Leu Val Ser Ile					
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Asn Asp Pro Met Leu Met Val Lys Lys Pro Ala Gly Leu Thr Ala Ala						
	180		185		190	
Thr Gly Met Asp Ala Leu Thr His Ala Ile Glu Ala Tyr Val Ser Lys						
	195		200		205	
Asp Ala Asn Pro Val Thr Asp Ala Ser Ala Ile Gln Ala Ile Lys Leu						
	210		215		220	
Ile Ser Gln Asn Leu Arg Gln Ala Val Ala Leu Gly Glu Asn Leu Glu						
225		230		235		240
Ala Arg Glu Asn Met Ala Tyr Ala Ser Leu Leu Ala Gly Met Ala Phe						
	245		250		255	
Asn Asn Ala Asn Leu Gly Tyr Val His Ala Met Ala His Gln Leu Gly						
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Gly Leu Tyr Asp Met Ala His Gly Val Ala Asn Ala Met Leu Leu Pro						
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His Val Glu Arg Tyr Asn Met Leu Ser Asn Pro Lys Lys Phe Ala Asp						
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Ile Ala Glu Phe Met Gly Glu Asn Ile Ser Gly Leu Ser Val Met Glu						
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Ala Ala Glu Lys Ala Ile Asn Ala Met Phe Arg Leu Ser Glu Asp Val						
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Gly Ile Pro Lys Ser Leu Lys Glu Met Gly Val Lys Gln Glu Asp Phe						
	340		345		350	
Glu His Met Ala Glu Leu Ala Leu Leu Asp Gly Asn Ala Phe Ser Asn						
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Pro Arg Lys Gly Asn Ala Lys Asp Ile Ile Asn Ile Phe Lys Ala Ala						
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Tyr						
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Primer

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Primer

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DATE: 04/24/2003

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3 <110> APPLICANT: SARCABAL, PATRICIA  
 4 CROUX, CHRISTIAN  
 5 SOUCAILLE, PHILIPPE  
 7 <120> TITLE OF INVENTION: METHOD FOR PREPARING 1,3-PROPANEDIOL BY A RECOMBINANT  
 8 MICRO-ORGANISM IN THE ABSENCE OF COENZYME B12 OR ONE OF  
 9 ITS PRECURSORS  
 11 <130> FILE REFERENCE: CHEP:004US  
 13 <140> CURRENT APPLICATION NUMBER: 10/043,639A  
 C--> 14 <141> CURRENT FILING DATE: 2003-04-12  
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 17 <151> PRIOR FILING DATE: 2000-07-07  
 19 <150> PRIOR APPLICATION NUMBER: FR 99/03939  
 20 <151> PRIOR FILING DATE: 1999-07-09  
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 28 <112> TYPE: DNA  
 29 <113> ORGANISM: Clostridium butyricum  
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 33 ttaaatgcta aacatgtgt tgaatcaga agagcaatat taataacaga atcattttaa 120  
 34 caaacagaag gacagcaga atttttaaga agagcatttg cattgaaaca catacttgaa 180  
 35 aatatcccta taacaattag agatcaagaa ctatagtgga gaagttttaa taaagaacca 240  
 36 aggtttttaa agtattttc tgagttttct aataagtggg tacaagatga attggataga 300  
 37 ttaataaaga gaattggaga tgcattccaa atttcagaag aaagtaaaaga aaatttaaaa 360  
 38 gatgtctttg agtatggaa tggaaagaca abaagtgggt tagcaacttc atatatgaca 420  
 39 taggaaacaa gaggagcagt aaattgtgaa gtatttaact taggaaacta ctattataat 480  
 40 ggcgaggagc atgtatctgt agattatgga aaagtattaa gggttggatt caatgggatt 540  
 41 ataattgagg ctagggaaca attagaaaaa aacaggagta tagatcctga ttttataaag 600  
 42 aaaghaaaat tctaaatag tcttattatc tcatggaga agtgcaataa atatgtaaat 660  
 43 agatattgcta aaagagctaa agagattgca gataatacaa gtgatgcaaa aagaaaagtt 720  
 44 gaattaaatg aaatagcaaa aatttgttca aaagtttcag gagagggagc taaatttttc 780  
 45 catgaagcat gtaattatt ttggtttatt catgcaataa taaatataga atctaattga 840  
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 47 aatataacag ataggtttgc tcaagaatta atagattgta tctggattaa attaaatgat 960  
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 53 acattagaag atgcaagaga ctacggaata attggatgtg ttgaacacaa aaagccagga 1320  
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57 catatgtgct gtgtgataa ttgcatagat attgcacatg cagaaaagagc tccattacct 1520
58 ttottgtcat caatgggtta taattgtatc ggaaaaggaa agagccttca agatgggtgg 1560
59 gcagaatata acttcagtgg accacaaggt gttggagtag ctaattattg agattcatta 1600
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63 tctagagaag ttaataaata tacaactcca aggggaggaa attttcaacc aggattatat 1760
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67 ttaatacaaa aattccatcc gtcagcatta aaaggtgata atggattaat gaattttaca 1920
68 tcaataataa caatttatct tcatcaaaa ggttttcatt tccaatttaa tctaatagat 1960
69 aaaaaaatat tacttgcagc acaaaaaaat cctgaaaaaat atcaagattt aattgctaga 2000
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2064

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76 &lt;212&gt; TYPE: DNA

77 &lt;213&gt; ORGANISM: Clostridium butyricum

79 &lt;400&gt; SEQUENCE: 2

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82 aatccagaat ccaagatat taaacctcaa gtaattgttt ataaaaattt atgtacaaaa 180
83 tctggaagat gtaaatctca atgtaaaagt gcaggtattg atatgaattc agaatatagg 240
84 atagataaaa gcaaatgtac agagtgtaca aaatgtgttg ataattgctt aagcggggga 300
85 cttgtttatt aaggaaggaa ttacagtgtt gaagacgtta taaagggaatt gaaaaaagat 360
86 agtghtcaat atagaagatc aaacgggtga attacactat ctggaggggg agtattactt 420
87 caacagatt ttgcagtga gcttttaaaa gagtgtaaat catatggctg gcacactgoc 480
88 attgaacacg caatgtatgt taatagtcaa tctgtaaaaa aagtaattcc atatatagat 540
89 ctggctatga ttgatataaa aagtlatgaat gatgaaaatc ataggaaaatt tacaggagtg 600
90 gttaacgaaa taattattaca aaacattaaa ttaagtgatg aattagctaa agaaataata 660
91 atcaqaattc ctggaataga aggatttaat gcagatttac aaagtatagg agcaatagct 720
92 caattttcaa aatcattaac aaactttaa agaatagac ttcttcata ccataattat 780
93 ggagaaaaata agtatcaagc aattggaaga gagtattctt tgaaagaaat aaaatcacct 840
94 agtaagaca aaatggaaag attaaaagct ttagttgaaa tcatgggaat accgtgcaca 900
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915

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97 &lt;210&gt; SEQ ID NO: 3

98 &lt;211&gt; LENGTH: 28

100 &lt;212&gt; TYPE: DNA

101 &lt;213&gt; ORGANISM: Clostridium butyricum

103 &lt;400&gt; SEQUENCE: 3

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107 &lt;210&gt; SEQ ID NO: 4

108 &lt;211&gt; LENGTH: 1158

109 &lt;212&gt; TYPE: DNA

110 &lt;213&gt; ORGANISM: Clostridium butyricum

112 &lt;400&gt; SEQUENCE: 4

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115 ttctcaaaag atatggaagg tggagctgtt gaattaacag ttaaatattt aaaagaagct 180
116 ggatttagatg ttgtatatta tgaaggagtt gaaccaaata caaaagatgt taatgtttata 240
117 ghaaggattaa aaatatattaa agaagaaaat tgtgacatga tagtaactgt aggtggagga 300
118 agtttagcatg attgoggtaa gggaatagga atttgtgcaa cacatgaagg agatctttat 360
119 gatttatgag gaatagaaac acttgtcaat ccattgcaac caatagtagc tgtaaatact 420
120 actgcaggaa ctgctagtga attaactcgt catttgtgtat tgaactaatc aaaaaagaaa 480
121 aaaaaatttg ttatagttag ctggagaagt ttgcctctag tatctatasa tgatccaatg 540
122 cttaigtgca aaaaaactgc aggattaaca gcagctacag gaatggatgc tttaacacat 600
123 gcaatagaag catatgtatc aaaagatgca aatccagtaa cagatgcttc agcaatacaa 660
124 gctattaaat taatttcaca aaatttaaga caagctgtag ctttaggaga aaatcttgaa 720
125 gcaagagaaa atatggctta tgcctcatta ctagcaggaa tggpatttaa taatgctaatt 780
126 tttagtatat tacatgcaat ggctcattca tttaggggac tgtatgatat ggccactggt 840
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135 <11> TYPE: DNA
136 <11> ORGANISM: Clostridium butyricum
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143 caattgtatt agttttaact ttagataaaa caaacaaaaa tgttattatt agccaagaaa 180
144 taactgttac aaaagaaaaa agaaaaacat agcaaaaagag taaccaatatt aagcaataaa 240
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147 agggctcaaa tattaaatgc taaaccatgt gttgaatcag aaagagcaat attaatatac 420
148 caatcattta aaaaaacaga aggcacagca gcaattttta gaagagcatt ggcattgaaa 480
149 cacataactg aaaaatatcc tataaccaatt agagatcaag aacttatagt gggaagttta 540
150 actaaagaac cagatgcttc acaagtattt cctgagtttt ctaataagtg gttacaagat 600
151 gaattggata gattaaataa gagaactgga gatgcattcc aaatttaaga agaaagttaa 660
152 gaaaaattaa aagatgtctt tgagtattgg aatggaaaag caacaagtga gttagcaact 720
153 tatatatga cagaggaaac aaaagatgca gtaaatgtg aagtatttac tgtaggaaac 780
154 tctattata atggcttagg acatgtatct gttagattat gaaaagtatt aagggttgga 840
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156 gattttataa agaaagaaaa attcctaaat agtgttatta tctcatgoga agctgcaata 960
157 atatatgtaa atagatatgc taaaaaggct aaagagattg cagataaatc aaaagatgca 1020
158 aaaaagaaaag ctgaatttaa tgaatatgca aaaaatttgt caaaagatac aggagagggg 1080
159 gtaaatctt tctatgaayc atgtcaatta ttttggttta tacatgcaat aataaatata 1140
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161 gaaatgata agaattattc agataagttt gctattaaat taatagattg taattggatt 1260
162 aatttaaatt atattaataa agtaagagat cagatttcaa ctacacatt tgggtgttac 1320
163 ctatgtatc aaaaattaat tgttgggggt caaaattcag aaggaaaaga tgcactaat 1380
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167 tctagaggto ttacattaga atatagcaga gactacggaa taattggatg tgttgaacca 1600
168 caaaagccag gaaaaacaga aggatggcat pattatgcac tttttaatct tgaagaataa 1600
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171 tatttttgaat aacatattgt ctgtctgat aatgataag atattgcaca tgcagaaaga 1600
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176 aaaaatgctc ctaagcttgg aatgataatt gatgaagttg ataatttagc tagagaggtt 1600
177 gcattagtat actgtagaga agttaataaa tatacaaatc caaggggagg aaattttcaa 1600
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199 tatggagaaa ataagtatca agcaattgga agagagtatt ctttgaaaag acaaaaatca 1600
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203 gagcaaaatt agtatcagta gtagggtgaaa gatgcacaaat attaggtgga aaaaaagcat 1600
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211 ctataaatga tccaatgtt atggtcaaaa aacttgcaag attaaagaa gtaagaggaa 1600
212 tggatctctt aatatactca atagaagcat atgtatcaaa agatgcacat ccagtaacag 1600
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216 atgatatggc atatgggtgt gctaattgaa tgcattatgc acatgttgaa cgttataata 4560
217 tgcattcaaa tcttaagaag ttggcagata tagcagaatt tatgggagaa aatataatctg 4620
218 gactttctgt aatggaagca gcagagaaag ccataaatgc aatgttcagg ctttcagagg 4680
219 atgttggaat tccgaaaagt cttaaaggaga tgggagtga acaagaagat tttgagcata 4740
220 tggcagaact agctctttta gatggaaatg ccttttagcaa tccaagaaaa ggaaatgcaa 4800
221 aagataattat aatatatttt aaggtctgctt attaattaat actatttaaa ggattccaaag 4860
222 taaaagataa aacatatata tattagattt aagattttat tataggctaa caacaaagaa 4920
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226 &lt;110&gt; SEQ ID NO: 6

227 &lt;111&gt; LENGTH: 783

228 &lt;112&gt; TYPE: PRT

229 &lt;113&gt; ORGANISM: Clostridium butyricum

231 &lt;400&gt; SEQUENCE: 6

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235 Ala Gln Ile Leu Asn Ala Lys Pro Cys Val Glu Ser Glu Arg Ala Ile
236 20 25 30
238 Leu Ile Thr Glu Ser Phe Lys Gln Thr Gly Gln Pro Ala Ile Leu Arg
239 35 40 45
241 Arg Ala Leu Ala Leu Lys His Ile Leu Glu Asn Ile Pro Ile Thr Ile
242 50 55 60
244 Arg Asp Gln Glu Leu Ile Val Gly Ser Leu Thr Lys Glu Pro Arg Ser
245 65 70 75 80
247 Ser Gln Val Phe Pro Glu Phe Ser Asn Lys Trp Leu Gln Asp Glu Leu
248 85 90 95
250 Asp Arg Leu Asn Lys Arg Thr Gly Asp Ala Phe Gln Ile Ser Glu Glu
251 100 105 110
253 Ser Lys Glu Lys Leu Lys Asp Val Phe Glu Tyr Trp Asn Gly Lys Thr
254 115 120 125
256 Thr Ser Glu Leu Ala Thr Ser Tyr Met Thr Glu Glu Thr Arg Glu Ala
257 130 135 140
259 Val Asn Cys Glu Val Phe Thr Val Gly Asn Tyr Tyr Tyr Asn Gly Val
260 145 150 155 160
262 Gly His Val Ser Val Asp Tyr Lys Val Leu Arg Val Gly Phe Asn Gly
263 165 170 175
265 Ile Ile Asn Glu Ala Lys Glu Gln Leu Glu Lys Asn Arg Ser Asp Pro
266 180 185 190
268 Asp Phe Ile Lys Lys Glu Lys Phe Leu Asn Ser Val Ile Ile Ser Cys
269 195 200 205
271 Glu Ala Ala Ile Thr Tyr Val Asn Arg Tyr Ala Lys Lys Ala Lys Glu
272 210 215 220
274 Ile Ala Asp Asn Thr Ser Asp Ala Lys Arg Lys Ala Glu Leu Asn Glu
275 225 230 235 240
277 Ile Ala Lys Ile Cys Ser Lys Val Ser Gly Glu Gly Ala Lys Ser Phe
278 245 250 255
280 Tyr Glu Ala Cys Gln Leu Phe Trp Phe Ile His Ala Ile Ile Asn Ile
281 260 265 270

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VERIFICATION SUMMARY

DATE: 04/24/2003

PATENT APPLICATION: US/10/043,639A

TIME: 16:21:34

Input Set : D:\Chep004.app

Output Set: N:\CRF4\04242003\J043639A.raw

L:14 M:271 C: Current Filing Date differs, Replaced Current Filing Date